

INTERNATIONAL COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Commissioner
 US Department of Commerce
 United States Patent and Trademark
 Office, PCT
 2011 South Clark Place Room
 CP2/5C24
 Arlington, VA 22202
 ETATS-UNIS D'AMERIQUE
 in its capacity as elected Office

Date of mailing (day/month/year) 13 December 2000 (13.12.00)	
International application No. PCT/GB00/01753	Applicant's or agent's file reference P23670A/RMC
International filing date (day/month/year) 08 May 2000 (08.05.00)	Priority date (day/month/year) 07 May 1999 (07.05.99)
Applicant BUTTLE, Louise, Georgina	

1. The designated Office is hereby notified of its election made:



in the demand filed with the International Preliminary Examining Authority on:

03 November 2000 (03.11.00)



in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was

was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Pascal Piriou Telephone No.: (41-22) 338.83.38
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PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:

MURGITROYD & COMPANY
373 Scotland Street
Glasgow G5 8QA
GRANDE BRETAGNE

17 SEP 2001

COMP

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing
(day/month/year)

14. 09. 01

Applicant's or agent's file reference

P23670A/RMC

IMPORTANT NOTIFICATION

International application No.

PCT/GB 00/ 01753

International filing date (day/month/year)

08/05/2000

Priority date (day/month/year)

07/05/1999

Applicant

EWOS LIMITED et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.
4. **REMINDER**
The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

** correction of cover sheet and annex pages
of IPEA dated 16.07.2001*

Name and mailing address of the IPEA/



European Patent Office
D-80298 Munich
Tel. (+49-89) 2399-0, Tx: 523656 epmu d
Fax: (+49-89) 2399-4465

Authorized officer


G. Hutterer
G. Hutterer



PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P23670A/RMC		FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB00/01753	International filing date (day/month/year) 08/05/2000	Priority date (day/month/year) 07/05/1999	
International Patent Classification (IPC) or national classification and IPC A23K1/18			
Applicant EWOS LIMITED et al.			
<p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 4 sheets, including this cover sheet.</p> <p><input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of <u>3 sheets</u></p>			
<p>3. This report contains indications relating to the following items:</p> <ul style="list-style-type: none"> I <input checked="" type="checkbox"/> Basis of the report II <input type="checkbox"/> Priority III <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability IV <input type="checkbox"/> Lack of unity of invention V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement VI <input type="checkbox"/> Certain documents cited VII <input type="checkbox"/> Certain defects in the international application VIII <input checked="" type="checkbox"/> Certain observations on the international application 			
Date of submission of the demand 03/11/2000		Date of completion of this report 16.07.2001	
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465		Authorized officer Graham, J Telephone No. +49 89 2399 7368	



INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/01753

I. Basis of the report

1. With regard to the elements of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17):*

Description, pages:

1-17 as originally filed

Claims, No.:

1-9 as received on 15/05/2001 with letter of 15/05/2001

Drawings, sheets:

1/4,2/4,4/4 as originally filed

3/4 as received on 15/05/2001 with letter of 15/05/2001

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB00/01753

- ☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1 - 9
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1 - 9
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1 - 9
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the International application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item I

Basis of the report

The amendments received on 15th May 2001 meet the requirements of Article 19(2) and 34(2)(b) PCT.

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

D1: IWAHASHI, M., AND WAKUI, H.: 'Intensification of Color of Fancy Carp with Diet' BULLETIN OF THE JAPANESE SOCIETY OF SCIENTIFIC FISHERIES, vol. 42, no. 12, 1976, pages 1339-1344, XP000939130

1.0 Novelty (Article 33(2) PCT)

1.1 The subject matter of claim 1 which relates to a method of enhancing the uptake of pigment by fish, whereby, the fish are fed with a pigment containing feed and 0.1 to 5% added cholesterol is deemed to be novel.

D1 explicitly states that the addition of cholesterol had no effect on the accumulation of carotenoids in fancy carp. Furthermore, the 0.07% cholesterol level of the feed in the trials carried out, is not within the scope claimed.

1.2 The above analysis applies mutandis mutatis to the subject matter of claim 5 and 9 which relates to the use of pigment containing fish feed to colour fish flesh, wherein, the feed contains 0.1 - 5% cholesterol.

2.0 Inventive Step (Article 33(3) PCT)

The subject matter of claims 1, 5 and 9 are considered as to involving an inventive step since D1 which acts as a technical prejudice, explicitly states that the addition of cholesterol to fish feed had no effect on the accumulation of carotenoids in fancy carp.

Re Item VIII

Certain observations on the international application

The wording "use of a fish feed containing pigment" in claim 5 has been interpreted to meaning "use of a pigment containing fish feed".

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P23670A/RMC	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/GB00/01753	International filing date (day/month/year) 08/05/2000	Priority date (day/month/year) 07/05/1999
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2. This REPORT consists of a total of 4 sheets, including this cover sheet.

☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 3 sheets

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand 03/11/2000	Date of completion of this report 16.07.2001
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Graham, J Telephone No. +49 89 2399 7368 

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB00/01753

I. Basis of the report

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**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB00/01753

- ☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

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6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

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	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1 - 9
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB00/01753

Re Item I**Basis of the report**

The amendments received on 15th May 2001 meet the requirements of Article 19(2) and 34(2)(b) PCT.

Re Item V**Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

D1: IWAHASHI, M., AND WAKUI, H.: 'Intensification of Color of Fancy Carp with Diet' BULLETIN OF THE JAPANESE SOCIETY OF SCIENTIFIC FISHERIES, vol. 42, no. 12, 1976, pages 1339-1344, XP000939130

1.0 Novelty (Article 33(2) PCT)

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D1 explicitly states that the addition of cholesterol had no effect on the accumulation of carotenoids in fancy carp. Furthermore, the 0.07% cholesterol level of the feed in the trials carried out, is not within the scope claimed.

1.2 The above analysis applies mutandis mutatis to the subject matter of claim 5 and 9 which relates to the use of pigment containing fish feed to colour fish flesh, wherein, the feed contains 0.1 - 5% cholesterol.

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The subject matter of claims 1, 5 and 9 are considered as to involving an inventive step since D1 which acts as a technical prejudice, explicitly states that the addition of cholesterol to fish feed had no effect on the accumulation of carotenoids in fancy carp.

Re Item VIII**Certain observations on the international application**

The wording "use of a fish feed containing pigment" in claim 5 has been interpreted to meaning "use of a pigment containing fish feed".

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 00/01753

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A23K1/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A23K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, FSTA, CHEM ABS Data, CAB Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	IWAHASHI, M., AND WAKUI, H.: "Intensification of Color of Fancy Carp with Diet" BULLETIN OF THE JAPANESE SOCIETY OF SCIENTIFIC FISHERIES, vol. 42, no. 12, 1976, pages 1339-1344, XP000939130 abstract; table 1	1-6,9-11
Y		7,8
Y	US 5 688 500 A (BARCLAY WILLIAM R) 18 November 1997 (1997-11-18) page 13, column 6, paragraphs 3-5; claims 1,4	7,8
	----- -/-	

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
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- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

G document member of the same patent family

Date of the actual completion of the international search

7 September 2000

Date of mailing of the international search report

21/09/2000

Name and mailing address of the ISA

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Authorized officer

Rooney, K

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 00/01753

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TYCZKOWSI, J. K., SCHAEFFER, J. L., AND HAMILTON, P. B.: "Influence of Dietary Lipids on Pigmentation of Young Chickens" POULTRY SCIENCE, vol. 68, no. 9, 1989, pages 1246-1254, XP000939108 cited in the application page 1252, column 2, paragraph 2 -page 1253, column 1, paragraph 1	1,11

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 00/01753

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5688500 A	18-11-1997	US 5518918 A	21-05-1996
		US 5340742 A	23-08-1994
		US 5340594 A	23-08-1994
		US 5130242 A	14-07-1992
		US 6103225 A	15-08-2000
		US 5908622 A	01-06-1999
		AU 711828 B	21-10-1999
		AU 4537497 A	19-02-1998
		AU 687016 B	19-02-1998
		AU 5327194 A	09-05-1994
		CA 2146235 A	28-04-1994
		EP 0669809 A	06-09-1995
		JP 11285376 A	19-10-1999
		JP 8502405 T	19-03-1996
		WO 9408467 A	28-04-1994
		US 5656319 A	12-08-1997
		US 5698244 A	16-12-1997
		AT 156520 T	15-08-1997
		AU 657259 B	09-03-1995
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		CA 2072978 A	18-05-1991
		DE 69031223 D	11-09-1997
		DE 69031223 T	05-03-1998
		DK 512997 T	02-02-1998
		EP 0512997 A	19-11-1992
		ES 2106736 T	16-11-1997
		GR 3025200 T	27-02-1998
		HK 1001922 A	17-07-1998
		JP 8509355 T	08-10-1996
		WO 9107498 A	30-05-1991

WO 00/67591

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18

1 CLAIMS

2

3 1. A method of enhancing the uptake of pigment by fish
4 to induce a change in the pigmentation of the flesh
5 by means of feeding the fish with cholesterol.
6

7 2. A method as claimed in Claim 1, wherein cholesterol
8 and pigment are added along with the fish feed.
9

10 3. A method as claimed in Claim 2 wherein the
11 cholesterol and/or pigment are components of the fish
12 feed.
13

14 4. A method as claimed in Claim 2 or 3 wherein the
15 cholesterol is provided in the same medium as the
16 pigment.
17

18 5. A fish feed for use in a method as claimed in Claims
19 1 to 4 wherein said fish feed comprises cholesterol
20 and pigment.
21

22 6. A fish feed as claimed in Claim 5 comprising between
23 0-5% cholesterol in the total feed.
24

25 7. A fish feed as claimed in Claim 5 or 6 wherein
26 cholesterol comprises between 1 - 4% of the feed.
27

28 8. A fish feed as claimed in Claim 5,6 or 7 wherein
29 cholesterol comprises between 1-3% of the feed.
30

WO 00/67591

PCT/GB00/01753

19

- 1 9. A fish feed as claimed in Claims 5 to 8 which can be
2 fed to any other fish species where the pigment
3 colour of the flesh or skin is important.
4
- 5 10. A fish feed as claimed in Claims 5 to 8 which can
6 be fed to Atlantic salmon, rainbow trout, tropical
7 fish, or any other fish species where the pigment
8 colour of either the flesh or skin is important.
9
- 10 11. The use of cholesterol to enhance the uptake of
11 pigment by fish.

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : A23K 1/18	A1	(11) International Publication Number: WO 00/67591 (43) International Publication Date: 16 November 2000 (16.11.00)
(21) International Application Number: PCT/GB00/01753 (22) International Filing Date: 8 May 2000 (08.05.00) (30) Priority Data: 9910461.4 7 May 1999 (07.05.99) GB (71) Applicant (for all designated States except US): EWOS LIMITED [GB/GB]; Ewos Technology Centre, Unit 1, Kingsthorpe Park, Houston Industrial Estate, Livingston EH54 5DB (GB). (72) Inventor; and (75) Inventor/Applicant (for US only): BUTTLE, Louise, Georgina [GB/GB]; 78 Harrison Gardens, Edinburgh EH11 1SB (GB). (74) Agent: MURGITROYD & COMPANY; 373 Scotland Street, Glasgow G5 8AQ (GB).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: PIGMENT		
(57) Abstract <p>The present invention provides a method of improving the pigmentation of fish flesh. Specifically, this is brought about through feeding the fish with both pigment and cholesterol, which are generally combined into a foodstuff. This addition of the pigments in the diet which results in a change in flesh colour, blood pigment levels and flesh pigment levels of the fish. Further, the uptake of pigment into the plasma and flesh is shown to be optimal when the feed contains a cholesterol level of between 1 and 3 percent. Such a method of enhancing the uptake of pigment by fish can be used on Atlantic salmon, rainbow trout, other salmonids, tropical fish and any other fish species where the pigment colour of either the flesh or skin is important.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
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1 "PIGMENT"

2

3 This invention relates to a method of altering and
4 improving the pigmentation of fish flesh. More
5 specifically the invention discloses a method of
6 enhancing the uptake of pigments by fish, such that
7 there is a resultant increase in the level of
8 pigmentation of the fish flesh.

9

10 An increase in market competition, coupled with the
11 widespread availability of fish in supermarkets has led
12 to an increase in the demand for, and quality of fish
13 products.

14

15 Mass production of salmonids such as salmon and trout
16 is required to meet current consumer demand which
17 exceeds that which can be met by fish produced in a
18 natural, wild environment.

19

20 Variations exist between fish produced naturally and
21 those which are specifically farmed to meet consumer

1 demand. One particular difference is a variation in
2 the colour of the flesh of the fish.

3
4 The characteristic pink colour of salmonid flesh is a
5 result of the deposition of naturally occurring
6 carotenoid pigments. Obtaining pigmentation in farmed
7 salmonids which is similar to that seen in wild salmon
8 is a vital aspect of feed production. Currently fish
9 feeds contain either or both of the main synthetic
10 pigments which are commercially available; astaxanthin
11 (Ax) and canthaxanthin (Cx). In several instances,
12 pigment costs contribute to 10-15% of the total cost of
13 fish feed production, compared to pigment flesh
14 deposition efficiencies which rarely exceed 15%. Since
15 fish feed comprises around 50% of the total production
16 costs of farmed fish, 5-7.5% of overall fish production
17 cost can be attributed to the cost of pigment.

18
19 Flesh colour is one of the main criteria used by the
20 consumer when considering the purchase of salmonids and
21 accordingly it is considered by the consumer that the
22 stronger red colour of the flesh which is seen in wild
23 fish is more desirable.

24
25 In an effort to achieve the flesh colour
26 characteristics exhibited by wild fish, pigments are
27 added to the feed given to farmed fish with the intent
28 that the uptake, by ingestion of the pigment, will lead
29 to an associated change in the colour of the flesh.

30

1 Pigments are specifically selected such that their
2 uptake will lead to the flesh becoming a red colour.
3 Examples of pigments which induce this are
4 canthaxanthin and astaxanthin.

5

6 Such processes are not limited to fish, as the
7 modification of the colour of naturally produced
8 foodstuffs is a current trend. The aesthetic appeal of
9 the product to the end consumer is enhanced through
10 modification of the feed ingredients to influence the
11 characteristics of the final product, in particular the
12 colour of the product.

13

14 An example of such a process currently known in the art
15 is the alteration of the feed ingredients given to
16 chickens and hens, such that the colour of the yolk of
17 the eggs that are produced is modified from that of the
18 natural colour. The result of this process is that the
19 product has an increased aesthetic appeal which in turn
20 leads to a greater desirability for consumer
21 consumption.

22

23 It is desirable for the flesh of the fish to be altered
24 to any specific requirement which may be set. One such
25 method of altering the fish flesh colour would be
26 through the introduction of pigments into the diet.

27

28 It is an object of the present invention to provide a
29 method for improving the uptake of pigments which are
30 provided in the diet to influence the colour of fish
31 flesh.

1 According to the present invention there is provided, a
2 method of enhancing the uptake of pigment by fish, the
3 method comprising feeding fish with cholesterol.

4

5 Preferably fish are fed cholesterol and pigment.

6

7 Preferably the cholesterol and/or pigment will be a
8 component of the fish feed.

9

10 Also preferably the cholesterol will be provided in the
11 same medium as the pigment.

12

13 Preferably the cholesterol will be added to the fish
14 feed at a level of between 0.1 to 5 percent.

15

16 Most preferably the cholesterol will be added to the
17 feed at a level of between 1 to 3 percent.

18

19 Preferably, the pigment will lead to a change in flesh
20 colour, plasma pigment levels and flesh pigment levels
21 of fish.

22

23 Preferably the method can be used on Atlantic salmon,
24 rainbow trout, other salmonid species, tropical fish.

25

26 Alternatively, the method may be used on any other fish
27 species where the pigment colour of either the flesh or
28 skin is important.

29

1 The invention also provides fish feed comprising
2 cholesterol and pigment.

3

4 The invention also provides the use of cholesterol to
5 enhance uptake of pigment to fish flesh.

6

7 Deposition of carotenoids in the fish flesh occurs as a
8 result of several processes; absorption of pigments in
9 the digestive tract, transport of pigment in the blood,
10 retention in the flesh and metabolism of carotenoids.

11 These processes are further detailed below;

12

13 1. **Absorption**

14

15 Pigment absorption across the intestinal wall,
16 from the digestive tract to the blood is the
17 initial phase in pigment retention by muscle in
18 salmonids. Since carotenoids are lipid soluble
19 they are most likely to be emulsified in a mixed
20 micelle together with bile, other lipid
21 components, prolipase and lipase during absorption
22 across the gastrointestinal tract (Leger 1985).

23

24 The rate of pigment absorption to the blood,
25 following ingestion is fairly slow, compared to
26 the absorption of essential fatty acids and amino
27 acids (Storebakken & No, 1992). Maximum plasma Ax
28 and Cx levels occurred at 24 hours following the
29 force feeding of rainbow trout with a 500mg dose
30 of Ax (March et al 1990, Choubert et al 1987),

1 carotenoid levels first being detected at 3 hours
2 following feeding.

3

4 2. **Blood Transport**

5

6 Ax and Cx are largely transported by the high
7 density lipoprotein fraction of plasma in immature
8 rainbow trout (Choubert et al, 1992, 1994).

9 Generally in immature fish, flesh is a major
10 tissue for storing carotenoids (No and
11 Storebakken, 1992).

12

13 3. **Deposition/Flesh Retention**

14

15 Deposition efficiency of dietary carotenoids in
16 salmonid flesh is in the range 1-18% (Torrissen et
17 al, 1989). Astaxanthin retention efficiency of
18 Rainbow trout was found to be significantly higher
19 than that for canthaxanthin; 11.4% and 7.1%
20 respectively (Storebakken & Choubert 1991). Dose
21 response studies show that the efficiency of
22 deposition declines with increase in dietary level
23 (50 mg/kg in Rainbow trout, Storebakken & No 1992:
24 10 mg/kg in Rainbow trout, Crampton 1995).

25

26 Differences in flesh retention efficiencies
27 between species have been observed, and it is
28 known that rainbow trout (RBT) pigment has a
29 greater efficiency than Atlantic salmon (ATS).

30

1 In the muscle of wild salmon (*Oncorhynchus keta*,
2 *O. nerka* & *O. kisutch*) astaxanthin (90% in the
3 free form) and canthaxanthin are bound to
4 actomyosin, probably via weak hydrophobic bonds
5 (Henmi et al 1987). Astaxanthin forms two
6 hydrogen bonds per one β ionone ring, and combines
7 more strongly than canthaxanthin, due to its
8 hydroxyl group (see Henmi et al 1989). The
9 actomyosin of salmon muscle can associate with
10 many kinds of carotenoids and lipids, implying
11 that specificity of binding sites is not a
12 problem, with variation between molecule types
13 relating to the bond strength (Henmi et al 1989).
14 In the skin the majority of astaxanthin is found
15 in the ester form (Torrissen et al 1989).

16

17 4 Metabolism

18

19 Carotenoids and their metabolites have been
20 detected in the tissues of fish up to 96 hours
21 following ingestion of a labelled meal (Guillou et
22 al 1992). Schiedt et al (1989) found idoxanthin
23 to be a metabolite of astaxanthin in ATS flesh -
24 higher levels of idoxanthin were found in
25 experimental fish in indoor tanks of farmed fish
26 in open cages, which suggests that this may be
27 stress related (Al-Khalifa & Simpson 1988).
28 Metabolites of carotenoids are found mainly in the
29 skin, but also in the flesh of sexually maturing
30 fish (Hata & Hata 1975; Scheidt et al 1985).

1 Schiedt *et al* (1985) evidenced that astaxanthin
2 could be a precursor to vitamin A in vitamin A-
3 depleted fish. Results of Al-Khalifa & Simpson
4 (1988) showed that astaxanthin was converted to
5 zeaxanthin, but in Vitamin A sufficient RBT it was
6 not converted to Vitamin A₁ and A₂ although fish
7 fed a diet lacking in vitamin A and carotenoids
8 for 30 days and then force fed astaxanthin showed
9 an increase in vitamin A.

10

11 This document suggests that the incorporation of a
12 pigment into the diet, either in combination with the
13 foodstuffs directly, or as a separate entity introduced
14 into the diet such that it will enter the same
15 metabolic pathways as other ingested and absorbed
16 nutrients will also end up as a constituent of the
17 flesh.

18

19 The pigment will lead to a change in the colour of the
20 flesh into which it is incorporated.

21

22 The incorporation of the pigment into the flesh may not
23 be efficient and this document identifies a method of
24 enhancing such pigment uptake.

25

26 The benefits of a method by which the uptake of pigment
27 by the fish is enhanced are wide-ranging and cover both
28 biological and economical aspects.

29

1 The addition of pigments such as astaxanthin and
2 canthaxanthin can have a drastic economical effect on
3 the cost of producing fish feed pellets, due to the
4 expensive cost of the pigments. As such a more
5 efficient mechanism of producing the effects of
6 astaxanthin and canthaxanthin may lead to a reduction
7 in the amount that needs to be added to the feed
8 initially.

9

10 Some research has indicated that lipid levels improve
11 pigment absorption for example Choubert et al (1991)
12 found that digestibility of Cx was greatly improved
13 when using a lipid rich diet (14% lipid/dry matter cf
14 4% lipid/dry matter). However, at commercially
15 realistic levels of lipid (24-35%) no differences were
16 found in flesh deposition efficiencies of RBT
17 (Crampton, 1996 internal data).

18

19 Bjerking et al (1997) found no significant effect of
20 dietary protein sources (eg a fish meal against a full
21 fat soyabean meal) in Atlantic salmon fed for 9.5
22 months, on the amount of astaxanthin in the muscle or
23 the visual colour score.

24

25 A study of biological utilisation of carotenoids (α and
26 β -carotene) in rats found that bioavailability of
27 naturally occurring carotenoids was greater than the
28 crystalline form (Tee et al 1996). In addition, Bierer
29 et al (1995) found that in pre-ruminant calves higher
30 serum levels of carotenoids were observed when given

1 commercial beadlet sources compared to crystalline
2 sources.

3

4 A Patent Application in the name of Finnfeeds
5 International Limited, (WO 9818345 A) claims that the
6 absorption in fish, crustaceans and healthy poultry of
7 pigments present in a non-viscous animal feed is
8 promoted by the presence of a carbohydrase and protease
9 enzyme.

10

11 In studies with young chickens Tyczkowski *et al* (1989)
12 found that lipids, long chain saturated fatty acids
13 (myristic, palmitic, stearic) and triglyceride,
14 tristearin, promoted minimal absorption of lutein,
15 whereas the short chain saturated lauric acid promoted
16 the highest absorption. Screening trials have been
17 conducted to try and identify enhancers of pigment
18 uptake that may be added to the feed to improve
19 pigmentation.

20

21 Cholesterol was tested as one of the enhancers, due to
22 its properties as an auxiliary agent in uptake.
23 Cholesterol is an important lipid in some membranes and
24 the plasma membranes of eucaryotic cells are usually
25 rich in cholesterol, this steroid also modulates the
26 fluidity of eukaryotic membranes. Due to these
27 properties cholesterol was identified as a substance
28 with the potential to enhance pigment uptake.

29

11

1 Cholesterol is added to the feedstuffs either by means
2 of extruder or via flex coating with a level of
3 addition between 0.5% and 5%. Natural levels of
4 cholesterol in commercial fish feeds (derived mainly
5 from fish oil) are up to approximately 0.5%.

6

7 In the same way that the pigmentation of salmonid
8 flesh, eg Atlantic salmon, Coho salmon, Chinook salmon,
9 Rainbow trout, Artic charr, is important to the
10 consumer, the skin colour of tropical fishes is also an
11 important quality characteristic. In this way the
12 feedstuffs of the above-mentioned species could be
13 modified in a similar way to effect the colour of flesh
14 and skin, in addition to flesh pigment concentration
15 (mg/kg).

16

17 A series of experiments are described below which look
18 at whether there is an enhancement of pigment uptake in
19 the plasma and flesh when the fish feed is supplemented
20 with varying levels of cholesterol.

21

22 Experiment 1

23

24 Atlantic salmon of mean weight 120g, were fed for a
25 period of 72 hours on one of two diets;

26

27 Diet 1: contains approximately 40ppm of canthaxanthin
28 (Cx)

29 Diet 2: contains 40ppm canthaxanthin (Cx) plus 0.48%
30 (total feed, 3% of the lipid coating phase) of
31 cholesterol.

12

1 Cx and cholesterol were added in the coating.

2

3 After feeding, fish were bled via the caudal vein,
4 using heparanised vacutainers, the blood samples were
5 centrifuged on site and the plasma removed and stored
6 frozen. Plasma pigment levels were analysed on HPLC.

7

8 Analysis results for the feeds are shown in Table 1.

9

10 **TABLE 1 Cholesterol levels in feeds**

11

Fish Feed	Cholesterol addition	% Cholesterol in feed
Uncoated feed	0	0.32
coated feed	0	0.27
coated feed	0.48%	0.53
coated feed	0	0.28
coated feed	0.48%	0.54

12

13

14 **TABLE 2 Plasma results for the treatmetns**

Replicate	Feed No.	Treatment	Cholesterol level % feed	Feed Cx mg/kg	Plasma Cx μ g/ml mean (STD)
1	1	CR	0	40.51	0.94 (0.5)
2					0.64 (0.4)
1	2	CR + Cholest-erol	0.48	45.67	1.42 (0.57)
2					1.45 (0.96)

15

16 significant differences were observed $p < 0.05$ (T-test)

1 CR = carophyll red (commercial formulation of Canthaxanthin)

2

3 The results shown in Table 2 clearly show that the fish
4 fed with cholesterol in feed (Diet 2) shown almost a
5 50% increase in the plasma Cx level compared to the
6 control feed. Additionally this trend is apparent in
7 both replicates of the experiments.

8

9 Experiment 2

10

11 Further experimentation investigating the effect of
12 supplementing dietary cholesterol on astaxanthin and
13 canthaxanthin flesh and plasma levels is described
14 below.

15

16 Atlantic salmon of an initial weight of 0.136Kg were
17 grown for four months in 12 x 3m tanks, supplied with
18 seawater. Fish were fed feeds containing varying
19 levels of cholesterol. (Sigma, C8503, approximately
20 95%). Cholesterol was mixed thoroughly with the oil
21 source and added in the coating (in addition to the
22 pigment preparations of astaxanthin (Ax) and
23 canthaxanthin (Cx)). Soya oil was selected as an oil
24 naturally low in cholesterol and this was the basis for
25 using fish foods with different oil source types and
26 the mixture of oils. Details of dietary cholesterol
27 levels and astaxanthin, canthaxanthin concentrations
28 are given in Table 3.

29

30 At the end of the trial, the fish were weighed, they
31 had their blood removed for pigment analysis, and flesh

1 samples scored with respect to colour and later
2 analysed for pigment.

3

4 The results of the experiments are further described
5 with reference to the figures wherein;

6

7 Figure 1 shows the effect of feed cholesterol
8 level on flesh pigment (Cx) concentration (mg/kg),
9 with each point on the figure representing the
10 mean value of each of the tanks,

11

12 Figure 2 shows the levels of the pigment
13 canthaxanthin in fish flesh, when cholesterol is
14 added to the feed effect of feed cholesterol level
15 (mg/kg), wherein each point is the mean value of
16 each tank (the 5 pooled analyses),

17

18 Figure 3 shows the effect of fed cholesterol level
19 on fillet SalmoFen scores and,

20

21 Figure 4 shows the effect of feed cholesterol
22 level on Minolta redness (a* value).

23

24 Figure 1 shows that the plasma pigment levels show an
25 increase which is correlated with an increase in
26 dietary cholesterol to approximately 1-3%. Any further
27 addition of cholesterol to the feed after this level
28 shows a decline in pigment plasma concentration.
29 Maximum canthaxanthin plasma level values were observed
30 at 3.6 $\mu\text{g/ml}$ (1% feed cholesterol added), compared to
31 control values of 1.5-2 $\mu\text{g/ml}$.

15

1 Figure 2 shows the effects on the levels of the pigment
2 canthaxanthin in fish flesh, when cholesterol is added
3 to the feed. Maximum flesh pigment levels of around
4 4.3 mg/kg were observed in the group of fish fed
5 canthaxanthin (which have a feed cholesterao level of
6 1.3%), compared to levels of around 3 mg/kg in the
7 control groups. In this size of Atlantic salmon,
8 dietary cholesterol levels (1-4%) caused an increase in
9 flesh pigment levels, this increase ranged from 0.4
10 mg/kg to 1.3 mg/kg.

11

12 Astaxanthin flesh levels were 2.32 mg/kg for the
13 control fish and 2.76 mg/kg for the fish with a 3.8%
14 cholesterol supplement to their feed. Astaxanthin
15 plasma levels were 0.62 $\mu\text{g/ml}$ for the control and 0.65
16 $\mu\text{g/ml}$ for the fish whose feed was supplemented with
17 3.8% cholesterol.

18

19 The effect of increasing the overall percentage of
20 cholesterol in feed with respect to the resultant
21 colour of the flesh is shown in Figure 3. The colour
22 is scored using a Roche SalmoFanTM score. This is a
23 tool used in the industry to score fish colour, which
24 was developed by Hoffman la Roche Ltd. The test
25 comprises a set of different coloured plastic mini
26 sheets which combine to form a scale that ranges from
27 20 (pale pink) - 34 (dark red), which are used to
28 compare against the colour of the fish flesh and score
29 them accordingly.

30

16

1 Maximum SalmoFan scores were observed with the tanks of
2 fish fed 1-2% cholesterol in the feeds. At higher feed
3 cholesterol levels, a decrease in Roche SalmoFan™
4 scores was observed (Figure 3). The difference in
5 flesh colour shown by the fish fed diets which had been
6 supplemented with between 1-2% cholesterol related to
7 1-1.5 points advantage on the Roche SalmoFan™ test.

8
9 Further analysis of the flesh colour was carried out
10 using the Minolta evaluation technique. Minolta redness
11 values are shown in Figure 2. The Minolta is a
12 tristimulus colorimeter (Minolta Chroma Meter CR300,
13 Minolta, Japan) which has an 8mm head and a D65 light
14 source. Readings were given for Lightness (L*),
15 Redness (a*) and yellowness (b*), the "L a b" system
16 according to International Commission on Illumination
17 (CIE, 1986). Maximum redness values were observed in
18 the fish fed which been supplemented with between 1-2%
19 of cholesterol, although the pattern was not as clear
20 as that exhibited by the results of the SalmoFan™
21 scoring system.

22
23 In conclusion, although the experiments described
24 herein show that the addition of any amount of
25 cholesterol to a fish feed at the level of 0 to 5% can
26 results in an increase in pigment levels in the plasma
27 and flesh, the results indicate that the optimum uptake
28 of pigment by the plasma and deposition in the flesh
29 occurs when the feed contains a cholesterol level of
30 between 1 to 3% of total feed weight.

1 Table 3:

Feed No	Cholesterol		Cholesterol Feed Level	Pigment Type	Dietary Pigment Conc (mg/kg)	Oil Source
	Feed Level (%)	Added (%)				
1441	Control	0.473	Cantha	55.11	fish oil	
1442	Control	0.382	Cantha	44.51	Fish/soya oil	
1443	Control	0.305	Cantha	50.94	Soya oil	
1444	1	1.258	Cantha	46.66	Fish/soya oil	
1445	2	2.186	Cantha	50.09	Fish/soya oil	
1446	3	3.142	Cantha	52.39	Fish/soya oil	
1661	4	4.001	Cantha	50.82	Fish oil	
1662	4	3.936	Cantha	53.47	Fish/soya oil	
1663	4	3.802	Cantha	48.62	Soya oil	
1664	Control	0.412	Asta	47.47	Fish/soya oil	
1665	4	3.803	Asta	44.86	Fish/soya oil	

1 CLAIMS

2

3 1. A method of enhancing the uptake of pigment by fish
4 to induce a change in the pigmentation of the flesh
5 by means of feeding the fish with cholesterol.

6

7 2. A method as claimed in Claim 1, wherein cholesterol
8 and pigment are added along with the fish feed.

9

10 3. A method as claimed in Claim 2 wherein the
11 cholesterol and/or pigment are components of the fish
12 feed.

13

14 4. A method as claimed in Claim 2 or 3 wherein the
15 cholesterol is provided in the same medium as the
16 pigment.

17

18 5. A fish feed for use in a method as claimed in Claims
19 1 to 4 wherein said fish feed comprises cholesterol
20 and pigment.

21

22 6. A fish feed as claimed in Claim 5 comprising between
23 0-5% cholesterol in the total feed.

24

25 7. A fish feed as claimed in Claim 5 or 6 wherein
26 cholesterol comprises between 1 - 4% of the feed.

27

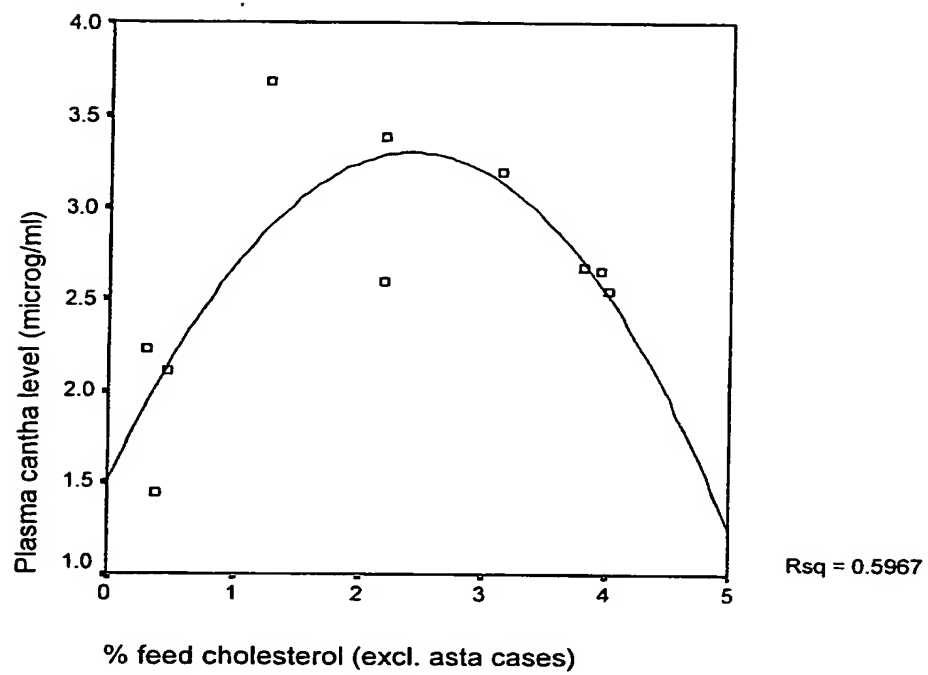
28 8. A fish feed as claimed in Claim 5, 6 or 7 wherein
29 cholesterol comprises between 1-3% of the feed.

30

- 1 9. A fish feed as claimed in Claims 5 to 8 which can be
2 fed to any other fish species where the pigment
3 colour of the flesh or skin is important.
4
- 5 10. A fish feed as claimed in Claims 5 to 8 which can
6 be fed to Atlantic salmon, rainbow trout, tropical
7 fish, or any other fish species where the pigment
8 colour of either the flesh or skin is important.
9
- 10 11. The use of cholesterol to enhance the uptake of
11 pigment by fish.

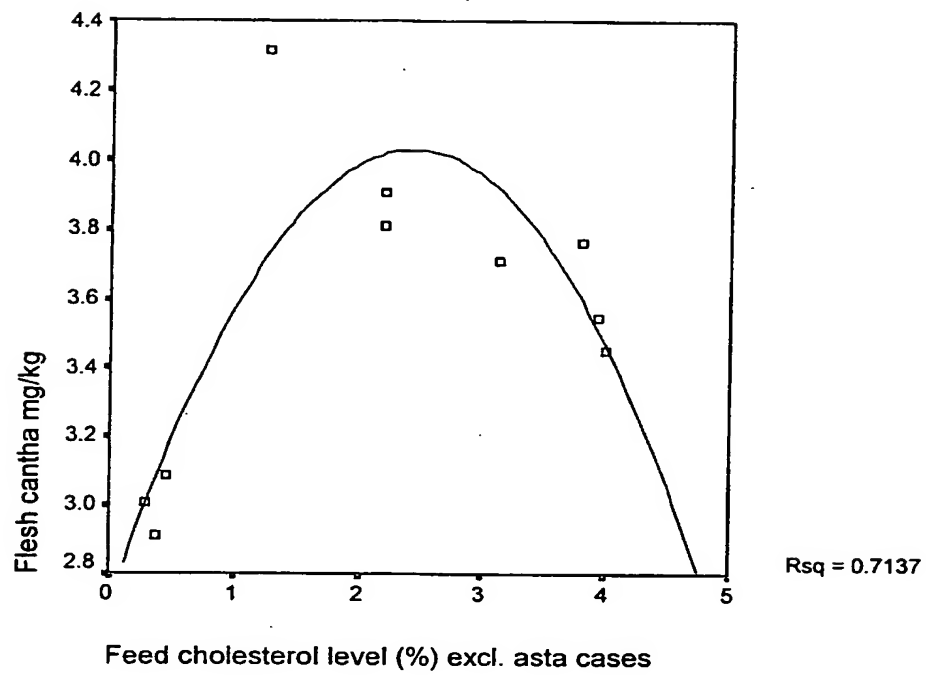
1/4

Figure 1: The effect of cholesterol feed level (%) on plasma cantha level in ATS ($\mu\text{g/ml}$)



2/4

Figure 2: The effect of cholesterol feed level (%) on flesh cantha level in ATS (mg/kg)



3/4

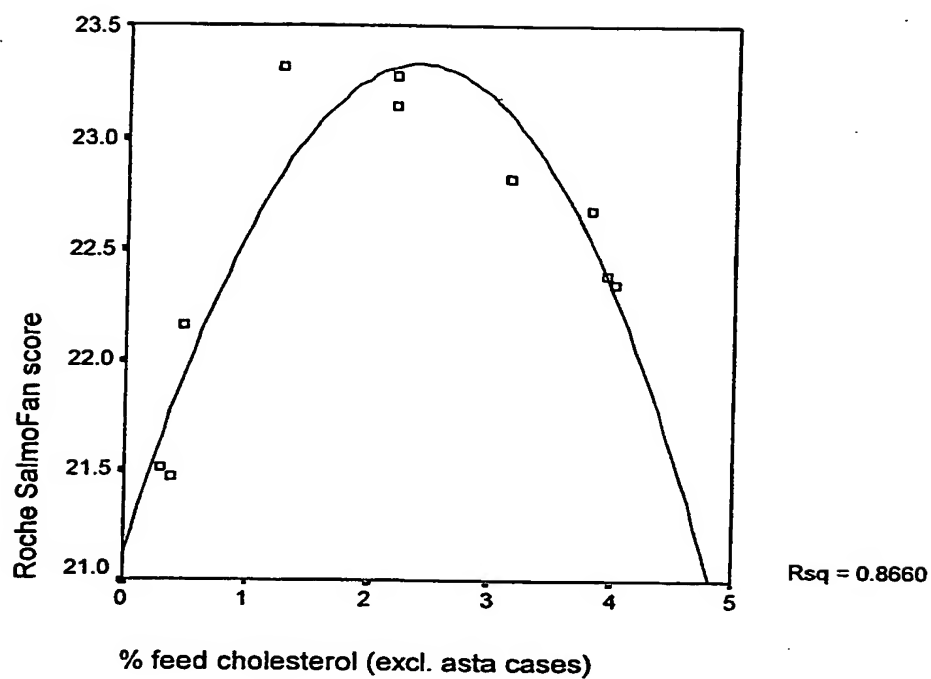
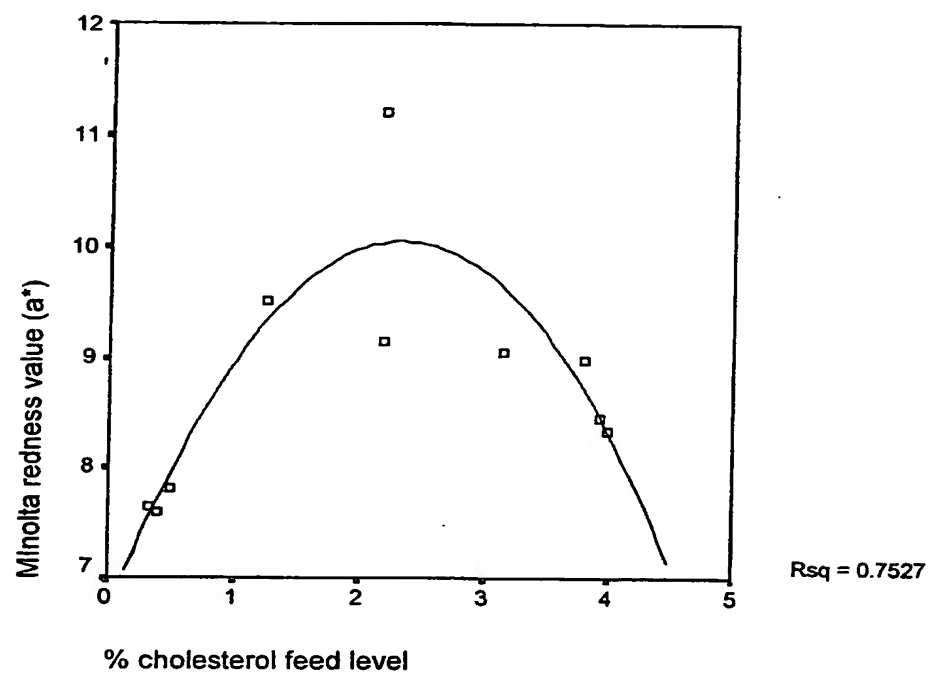


Figure 3: The effect of cholesterol feed level (%) on plasma cholesterol level in ATS (g/l)

4/4

Figure 4: The effect of feed cholesterol level on minolta redness (a^*) value

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference P23670A/RMC	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/GB 00/ 01753	International filing date (day/month/year) 08/05/2000	(Earliest) Priority Date (day/month/year) 07/05/1999
Applicant EWOS LIMITED et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☒ None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No.

T/GB 00/01753

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A23K1/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A23K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, FSTA, CHEM ABS Data, CAB Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	IWAHASHI, M., AND WAKUI, H.: "Intensification of Color of Fancy Carp with Diet" BULLETIN OF THE JAPANESE SOCIETY OF SCIENTIFIC FISHERIES, vol. 42, no. 12, 1976, pages 1339-1344, XP000939130 abstract; table 1	1-6,9-11
Y	---	7,8
Y	US 5 688 500 A (BARCLAY WILLIAM R) 18 November 1997 (1997-11-18) page 13, column 6, paragraphs 3-5; claims 1,4 --- -/--	7,8

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

7 September 2000

Date of mailing of the international search report

21/09/2000

Name and mailing address of the ISA

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Authorized officer

Rooney, K

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 00/01753

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TYCZKOWSI, J. K., SCHAEFFER, J. L., AND HAMILTON, P. B.: "Influence of Dietary Lipids on Pigmentation of Young Chickens" POULTRY SCIENCE, vol. 68, no. 9, 1989, pages 1246-1254, XP000939108 cited in the application page 1252, column 2, paragraph 2 -page 1253, column 1, paragraph 1 -----	1, 11

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 00/01753

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5688500 A	18-11-1997	US 5518918 A	21-05-1996
		US 5340742 A	23-08-1994
		US 5340594 A	23-08-1994
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